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Case report

Biomarkers of recent drinking, retrograde extrapolation of blood-alcohol concentration and plasma-to-blood distribution ratio in a case of driving under the influence of alcohol

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ABSTRACT

This case report describes the police investigation of a road-traffic accident involving a collision at night (01.00 am) between a car and a truck in which a passenger in the car was killed. The driver of the truck was found responsible for the crash although a roadside breath-alcohol test was negative (<0.1 mg/L breath or 20 mg/100 mL blood). Because of injuries sustained in the crash, the female driver of the car was not breath-tested at the time but was transported to a local hospital for emergency treatment. After swabbing the skin with isopropanol an indwelling catheter was inserted at 01.40 am. A blood sample was taken at 02.10 am and the plasma portion contained 8 mmol/L ethanol according to analysis at the hospital clinical laboratory using a gas chromatographic method. Another blood sample was taken at 05.45 am for analysis of ethanol at a forensic toxicology laboratory, although the result was negative (<10 mg/100 mL). The police authorities wanted an explanation for the discrepancy between the clinical and forensic laboratory results and inquired whether the driver of the car was above the legal alcohol limit (>20 mg/100 mL) at the time of the crash. The scientific basis for converting a plasma-ethanol concentration into a blood-ethanol concentration and back extrapolation of the driver's blood-alcohol concentration (BAC) is explained. The risk of contaminating a blood sample by swabbing the skin with isopropanol is discussed along with the use of alcohol biomarkers (ethyl glucuronide and ethyl sulphate) as evidence of recent drinking.

1. Introduction

Driving under the influence of alcohol is a global public health problem and impaired drivers are responsible for many road-traffic crashes and deaths on the roads.[1,2] Most nations enforce statutory concentration limits of alcohol in blood, breath or urine above which it is an offence to drive.[3] However, a person's blood-alcohol concentration (BAC) is constantly changing both during and after drinking and the final result depends on many environmental and biological factors.[4] The importance attached to the driver's BAC for a successful prosecution means that results reported are sometimes challenged, owing to faulty police procedures, alleged problems with analysis of the samples or issues related to the disposition and fate of ethanol in the body.[5,6]

In Sweden, like many other countries, the BAC *per se* constitutes whether an offence was committed and evidence that the driver was impaired by alcohol is not necessary for a successful prosecution.[7] Moreover, in Sweden the driver's BAC during or after driving is the value used for prosecution, whereas in other countries it is the BAC at the time of driving or within a reasonable time afterwards.[8] What constitutes a reasonable time is open to discussion but 2–3 h after the driving is accepted in Canada and many US states. If a blood sample is obtained beyond this time the prosecution is required to perform a back extrapolation of BAC to the time of driving, which is a dubious practice.

Statutory blood-alcohol limits for driving refer to the concentration of ethanol determined in a specimen of whole blood and not in plasma (P) or serum (S), which are the biological specimens analyzed at hospital clinical laboratories.[9–11] Under some circumstances, a driver might be injured in a traffic crash and taken to hospital for emergency treatment, which means that the concentration of alcohol determined in plasma or serum finds its way into evidence in a drunken driving case.[11] Because the concentration of alcohol in plasma or serum is higher than in an equal volume of whole blood, the hospital laboratory result should not be used for prosecution in road-traffic cases without an appropriate correction being made. This requires information about the distribution of ethanol between plasma and whole blood and the magnitude of

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inter- and intra-individual variations in this important ratio.[12,13] In this connection gender- and disease-related variations in haematocrit and water content of blood and the lipid content of plasma deserves careful consideration.[14,15]

2. Case report

This case report deals with a collision between a car and a truck in which a passenger in the car was killed. Responsibility for the crash was attributed to the driver of the truck because he had attempted to make a U-turn on a highway at night. The occupants of the car were returning from vacation and their flight landed at the International airport in Stockholm at 10.00 pm. The crash occurred at 1.00 am during the drive home, a trip of about 250 km. A male passenger travelling in the car was pronounced dead at the scene when police and ambulance personnel arrived at 01.18 am and 01.24 am, respectively.

The result of a roadside breath-alcohol test made on the driver of the truck was negative (<0.10 mg/L breath or <20 mg/100 mL blood). The driver of the car was not breath-tested at the time of the crash because of injuries sustained and was later transported to hospital for emergency treatment. At 01.40 am paramedics inserted an intravenous catheter in a vein on the arm of the driver of the car after swabbing the skin with isopropyl alcohol (70% v/v).

On arrival at the hospital at 02.10 am a blood sample was taken via the catheter and an aliquot of plasma was sent for analysis to the hospital clinical laboratory. Besides the usual biochemical and haematological analytes, an aliquot of plasma was analyzed for alcohol and other volatiles by gas chromatography. The concentration of ethanol in plasma was reported as 8 mmol/L. The police requested that a specimen of blood was obtained for analysis at a forensic laboratory and this was done at 05.45 am without use of a skin disinfectant using grey-stopper evacuated tubes containing sodium fluoride as a preservative. The blood-ethanol concentration was determined by headspace gas chromatography and reported as negative using a limit of quantitation (LOQ) of 10 mg/100 mL.

The police asked for an explanation about the discrepancy between the hospital laboratory result (plasma-ethanol 8 mmol/L) and the negative result from analysis of blood at the forensic laboratory. They wanted to know if the concentration in plasma could be converted into BAC and whether the driver of the car was above the legal limit at the time of the crash. They also wondered about use of isopropanol to swab the skin before inserting the catheter and whether this might have contaminated the blood specimen and invalidated the analytical result from the clinical laboratory.

There are a number of interesting forensic issues arising in this case;

- Conversion of plasma-ethanol concentration to whole blood-ethanol concentration.
- Retrograde extrapolation of BAC from time of sampling blood to time of driving.
- Risk of contaminating the blood sample by swabbing the skin with isopropanol.
- The application of alcohol biomarkers as evidence of recent drinking even when BAC was negative.

3. Results and discussion

The time-line of events in this case from arrival at the airport to starting the drive home until time of the collision and sampling of blood for determination of alcohol are summarized in Table 1.

3.1. Plasma/blood-alcohol ratio

If the concentration of ethanol determined in plasma (8 mmol/L) is taken at face value, this can be converted into the concentration in whole blood based on published studies of the plasma-to-blood distribution ratio of ethanol. It should be borne in mind that unlike the situation in forensic laboratories where duplicate determinations are made, clinical laboratories only make single determinations and no allowance is made for uncertainty in the method used.[16] Furthermore, chain-of-custody procedures in clinical laboratories are not as rigorous as they are in forensic laboratories. Nevertheless most clinical laboratories participate in external proficiency tests and this includes determinations of ethanol so information about past performance of the actual laboratory is available on discovery.[17]

The distribution ratio of alcohol between plasma and whole blood has been measured in several studies over the years and the results depend on relative water content of plasma and erythrocytes.[11–13] Accordingly, the concentration of ethanol in plasma is expected to be about 10–20% higher than in an equal volume of whole blood.[12] Note that the serum/blood distribution ratio of

Table 1
Time-line of events leading up to a collision between a truck and a car in which a passenger in the car was killed and the estimated blood-alcohol concentration (BAC) of the driver of the car.

Time of day	Description of event before and after the crash	Conservative estimate of the driver's BAC
10.00 pm	A man and woman were returning from vacation and landed at the international airport in Stockholm.	73 mg/100 mL[a]
10.30 pm	The couple started their drive home, a distance of about ~250 km, with the women at the wheel of the car and her male companion a passenger in the front seat.	68 mg/100 mL[a]
01.00 am	A collision occurred when the driver of a truck attempted to make a U-turn on the highway at night. The result of a roadside breath-alcohol test on the driver was negative.	43 mg/100 mL[a]
01.18 am–01.24 am	Police and ambulance personnel arrived at the crash scene. The male passenger in the car was pronounced dead although the driver was not breath-tested owing to injuries sustained in the crash.	40 mg/100 mL[a]
01.40 am	An indwelling catheter was inserted in a superficial vein on the arm of the car driver after swabbing the skin with isopropanol. The driver of the car was then taken to hospital.	36 mg/100 mL[a]
02.10 am	Blood was sampled from the catheter on arrival at the hospital and the plasma portion separated and analyzed for volatiles by a gas chromatographic method.	Plasma-ethanol was 8 mmol/L, which corresponding to a blood-ethanol of 31 mg/100 mL.[b]
05.45 am	A venous blood sample was taken using evacuated tubes and sent for analysis to a forensic toxicology laboratory.	Blood-alcohol concentration negative (<10 mg/100 mL).[c]

[a] Derived using a low rate of alcohol elimination from blood of 10 mg/100 mL per h.
[b] Calculated using a plasma-to-blood distribution ratio of ethanol of 1.2:1 (mean + 2.57 SD).
[c] Method LOQ was 10 mg/100 mL for reporting positive results, although biomarkers of recent drinking were positive (see text for details).

ethanol is the same as the plasma/blood distribution ratio, because plasma and serum contain the same amount of water.[18] Moreover, the pharmacokinetics of ethanol can be studied based on samples of plasma or whole blood and the absorption, distribution and elimination stages of the curve can be characterized regardless of the type of specimen analyzed.[19]

The most comprehensive study of the serum/blood distribution of water was published by Iffland et al.[20] These investigators determined the water content of serum and blood in $N = 833$ paired samples pooling the results from three different laboratories. The overall mean distribution ratio of water was 1.16:1 with a standard deviation (SD) of 0.0163. This distribution of water will be the same as the distribution of ethanol between plasma or serum and whole blood. Assuming a Gaussian distribution of serum/blood ratios, it follows that 99% of individuals in the same population will have a ratio varying from 1.12:1 to 1.20:1 (mean $\pm$ 2.57 $\times$ SD). Only one person in 200 is likely to have a plasma/blood ratio above 1.2:1. However, in any individual case, the plasma/blood distribution ratio will depend on age and gender and especially the water content and haematocrit of the blood sample as well as medical conditions, such as anaemia and polycythemia.[14,15]

The plasma sample taken at 02.10 am from the driver of the car contained 8 mmol/L ethanol, which corresponds to a concentration in whole blood of 6.7 mmol/L (8.0/1.2 = 6.7 mmol/L). When these SI units are translated into mass/volume units the woman's BAC at 02.10 am becomes 31 mg/100 mL (6.7 $\times$ 46)/10)), where 46 is the molecular weight of ethanol.

3.2. Back extrapolation

The elimination rate of alcohol from blood in humans was the subject of a recent evidence-based review article and a range of 10–25 mg/100 mL per hour was suggested for the vast majority of people.[21] According to the police report the driver of the car had not consumed any alcohol since lunchtime the day before the crash. This gives compelling evidence that the post-absorptive phase of the blood-alcohol curve existed at the time of the crash (01.00 am), and at the time of sampling blood (02.10 am) and even when starting to drive home from the airport (10.30 pm). A back extrapolation is therefore motivated and in this calculation, to give the suspect any benefit of the doubt, a relatively low rate of ethanol elimination from blood should be used, such as 10 mg/100 mL per hour.[21]

The driver's BAC at 02.10 am according to analysis of plasma was 31 mg/100 mL (see above) and at the time of the crash 70 min earlier (01.00 am) by back extrapolation a BAC of 43 mg/100 mL is obtained. On arrival at the airport in Stockholm (10.00 pm) the woman's BAC was considerably higher (73 mg/100 mL) and when starting to drive home this had decreased to 68 mg/100 mL (Table 1). These estimates are conservative because of the low rate of ethanol elimination used in the back extrapolation.[21]

3.3. Swabbing the skin with isopropanol

The question of swabbing the skin with alcohol and the risk of contaminating a blood sample taken directly afterwards has been tested experimentally on several occasions.[22–26] Isopropanol was the disinfectant used in this case and not ethanol. These two alcohols are separated when the blood is analyzed by gas chromatography so the possibility of elevating the concentration of ethanol is unfounded.[27] Even if the skin had been swabbed with ethanol, studies have shown that risk of contaminating the blood when using evacuated tubes to take the samples is virtually non-existent.[22–26]

3.4. Biomarkers of alcohol consumption

The driver of the car had admitted daily consumption of alcohol during vacation and the last drink was taken about 12 h before the crash occurred. The discrepancy between the negative BAC report from the forensic laboratory and 31 mg/100 mL based on analysis of plasma at the clinical laboratory is not unexpected since ~3½ h has elapsed between taking the samples. During this time ethanol is eliminated from the body through metabolism.

Nevertheless, it was of interest to investigate whether there were biomarkers of recent drinking in the forensic blood sample obtained at 05.45 am.[28] To this end arrangements were made to analyse ethyl glucuronide (EtG) and ethyl sulphate (EtS), which are non-oxidative metabolites of ethanol, and can be used to disclose recent drinking even after ethanol is no longer measurable in blood or urine samples.[29] EtG and EtS were analyzed in blood by LC-MS at an independent laboratory and the concentrations were 11.0 mg/L and 3.7 mg/L, respectively compared with expected values of zero. These two alcohol biomarkers verify that the women had some time earlier consumed alcohol despite a negative BAC reported by the forensic laboratory.

4. Conclusions

This report illustrates some of the questions that might arise during police investigations of road-traffic crashes involving alcohol-impaired drivers and when culpability for the crash is determined. Various aspects of the interpretation of a measured blood-alcohol concentration are highlighted including conversion of plasma-ethanol into blood-ethanol. The magnitude of inter- and intra-individual variations in plasma/blood distribution ratio should be considered when this conversion is made.[20]

The elimination rate of alcohol from blood is usually not known in any individual and the value used in the back calculation should therefore be conservative in criminal cases, such as 10 mg/100 mL per h.[20] The existence of the post-absorptive phase of the blood-alcohol curve at the time of the crash and the time of sampling blood is also something that needs to be explained to the court.[30–34] The various scientific studies relied upon when expert testimony is rendered should be supported by reference to articles published in peer reviewed scientific journals.

At the time of the crash the driver of the car had a BAC of at least 43 mg/100 mL, which is more than twice the legal limit for driving in Sweden (20 mg/100 mL). When leaving the airport the driver's BAC was at least 68 mg/100 mL. Despite forensic evidence pointing towards a punishable BAC in the female car driver at the time of the crash, the crown prosecution service in Sweden decided not to prosecute because responsibility for the crash had already been attributed to the driver of the truck.

Ethical approval

No application was made for ethical approval to write this case report because it is not possible to identify anyone involved.

Conflict of interest

None declared.

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